Effects of Anthropogenic Noise on Soundscape and the Stress Hormone Levels of the Gulf Coast Toad (*Incilius valliceps*) in Central Texas

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Abstract:

Environmental stressors affect all organisms and those stressors play a role in driving adaptive evolution. Stress may reduce reproductive success or suppress immune system responses, thus reducing fitness (Bee & Swanson 2007; Wollermann & Wiley 2002; Parris et. al. 2009; Rollinns-Smith et. al. 2011; Gabor et. al. 2013). One such stressor includes sound (Tennessen et. al. 2014), with variation in frequency, intensity, and source influencing how stressful a specific sound is for a particular species. All the sounds present within an environment make up a soundscape. With increasing fragmentation due to roads and highways, populations are becoming increasingly subjected to noise from traffic; particularly on the borders of the fragmented habitats. To understand the role road traffic noise plays in a soundscape we collected 123 sound samples within a wilderness preserve bordered by a major highway. Our results indicate that distance from the highway can explain 66.1% of noise variation within the preserve. Recent studies have shown that road noise does affect anuran behavior primarily in regards to mate choice (Bee & Swanson 2007; Wollermann & Wiley 2002; Parris et. al. 2009). Road noise may also indirectly affect reproduction if it triggers chronic stress. Therefore, we inquire if proximity to road noise is correlated with variation in corticosterone concentrations for a population of Gulf Coast toad (*Incilius valliceps*) in Wild Basin Wilderness Preserve, Austin, Texas. To answer this question we will quantify urinary corticosterone levels in toads, measure their distance from TX-360, and quantify the noise levels across the preserve.

Introduction:

Global biodiversity decline can strongly be attributed to human alteration of environments. As human populations increase, ecosystems often become increasingly fragmented, disturbed, and polluted. Chemical pollutants like pesticides or byproducts from fossil fuel consumption easily escape from urban environments into natural ecosystems. Amphibians are commonly among the first organisms to be affected by pollution deeming them bioindicators. Bioindicators are organisms that reveal the health of an ecosystem through extinction or decreased population size within an ecosystem. A drastic decrease in a local amphibian population can indicate a polluted environment, acting as a signal for overall environmental health. A population exposed to pollutants may be under stress from poor environmental conditions and may be more susceptible to infectious pathogens, such as chytrid fungus due to stress-induced immune suppression (Rollins-Smith et. al. 2011; Carey et. al. 1999), or reduced growth rates from pollutants (Peterson et. al. 2008). Anurans have recently seen global population and species decline due to outbreaks of chytrid fungus (*Batrachochytrium dendrobatidis*), which infects the skin of immunosuppressed individuals (Rollinns-Smith et. al. 2011; Gabor et. al. 2013). Therefore, the measure of stress levels in a population of anurans can potentially be used as a bioindicator. Ultimately, reducing the effects of anthropogenic induced stress may help in the conservation of species diversity.

Corticosterone:

Stress is most commonly measured by sampling the concentration of glucocorticoids, including corticosterone, within an organism. Corticosterone is a type of glucocorticoid produced by the body constantly throughout the lifespan of many organisms including mammals, birds, reptiles, and amphibians. During periods of stress corticosterone levels can increase significantly. Common stressors include hunger and perceived risk of predation as well as pollution. When a stressor is perceived by the brain, the hypothalamus induces the creation of corticotrophin releasing hormone (CRH) that in turn triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH then triggers the release of corticosterone from the adrenal glands (Goodenough et. al. 2010c). Once introduced into the system, the hormone induces the release of glucose to the blood to provide the energy needed for the organism to react to its stressor.

This reaction is necessary for the survival of an organism, and short term or acute stress generally does not have permanent physiological effects. It allows for the animals to have the energy to escape predation or search for resources. However, long term or chronic stress effects can have a significant impact on the organism or population level. Chronic stress may reduce growth rates (Arendt 1997; Goodenough et. al. 2010a), reproductive rates (Scheuerlein et. al. 2001; Goodenough et. al. 2010a), and the effectiveness of the immune system (Goodenough et. al. 2010c). Chronic stress may even affect the morphology of growing organisms (Goodenough et. al. 2010a). Maher et. al. (2013) showed that long term treatment of tadpoles with corticosterone was correlated to longer tails of tadpoles. This is an irreversible change that may potentially affect the organism's' overall fitness. In

addition, tadpoles present in drying ponds had higher levels of corticosterone and metamorphose significantly more rapidly under those conditions (Denver 1998; Denver et. al. 1998). Thus, stress and corticosterone play a major role in morphological development, reproductive success, and immune system function of anurans.

Soundscape:

The source of stress we analyzed here is noise and its effect on soundscape. A soundscape is defined by the variation of sound intensity or frequencies within a landscape. It is well known that acoustics play a major role in the life history of many frog and toad species, as calling is how many find mates (Goodenough et. al. 2010b). Mate calling is energetically costly and success dictates the passage of an individual's genes onto the next generation. Hindering the effectiveness of those calls may also hinder reproduction possibly altering the overall abundance of the population.

For example, road traffic noise has been shown to affect some female frogs' ability to locate the source of their calling mates (Bee & Swanson 2007). Background noise also affects female preference in frequency of calls (Wollermann & Wiley 2002). In addition, wave frequency of calls from males is altered by traffic noise (Parris et. al. 2009). These studies show the significance of acoustic anthropogenic disturbance to anurans. Here we investigate how anthropogenic noise affects soundscape and its subsequent impact on the urinary corticosterone levels in the Gulf Coast toad (*Incilius valliceps*). Laboratory experiments support that recorded road traffic noise can alter corticosterone levels in

anurans (Tennessen et. al. 2014). We ask if road traffic noise can alter stress hormone levels in natural settings.

Hypotheses:

We predict that sound intensity (measured in decibels; db) will decrease as distance from a major highway increases. Sound intensity is also expected to have a positive correlation to *Incilius valliceps* urinary corticosterone concentrations. Our third hypothesis predicts *I. valliceps* corticosterone concentrations to decrease as distance from the highway increases.

Methods:

Study Site:

This study will be conducted at Wild Basin Wilderness Preserve in Austin, Texas. Wild Basin Preserve was founded in 1974. Wild Basin contains around 92 hectares of land. It is part of the larger Balcones Canyonlands Preserve (BCP) system, promoting the protection of eight endangered species and twenty-seven species of concern. Wild Basin is open to the public for trail hiking and events, though its main purpose is to protect habitat for the endangered golden-cheeked warbler. Bikes, pets, smoking, and picnics are all prohibited within the preserve (Wild Basin Creative Research Center 2015).

Wild Basin is adjacent to the Capital of Texas Highway or Texas State Highway Loop-360 (TX-360). The majority of TX-360 that is along Wild Basin has two lanes on either side. The Capital of Texas Highway was opened in 1970 and is 22.51 km long. It is the 98th most

crowded road in Texas. TX-360 is reported to have greater than 155,000 hours of time delay per mile annually. A large portion of these hours are accumulated by rush hour traffic, which causes travel time to take 60 percent longer than normal traffic (Texas Department of Transportation 2015). Rush hour occurs twice a day; in the morning from 6:00am to 10:00 am and evening from 3:00pm to 7:00pm. Therefore, we chose to take our sound measurements during a rush hour time period.

Soundscape:

A soundscape is defined by the variation of sound within a landscape. In this study we started by defining the soundscape of the Wild Basin Wilderness Preserve. We recorded sound levels measured in decibels on Tuesdays, Thursdays, and Fridays between 4:00 and 6:00 PM, during the height of evening rush hour. This was performed by walking throughout the preserve in a grid pattern while using a smartphone application with a microphone to record sound intensity every one hundred meters or so. We also did some strategic sampling to get a good representation of elevation in the preserve. The application and microphone are Occupational Safety and Health Administration (OSHA) approved (Kardous & Shaw 2014). The application was acquired through iTunes and is called SoundMeter. The microphone was a MicW i436 microphone. Each sound recording was thirty seconds long. The application gave us maximum, peak, and average decibel readings at each location. To find the coordinates of each location we used Backpacker GPS Trails Lite. This application was chosen because it had very high reviews and instead of using your phone's positioning device it connects to satellites like a hand held GPS unit. We

also recorded the time and weather conditions. We acquired a total of 123 data points. We entered all of the data into ArcGIS. We then used the near analysis tool to find the distance of each sound coordinate from the Capital of Texas Highway. Once the distance in meters was acquired a regression analysis was ran on the data using SPSS statistical software. We also used ArcGIS to create a color gradient map to show the variation in average sound intensity throughout the preserve.

Study Species:

The species chosen for this study was the Gulf Coast toad, *Incilius valliceps.* This species was formerly known as *Bufo valliceps*. It is distributed along the coast of the Gulf of Mexico, starting in southern Louisiana and continuing down the coast until it reaches Costa Rica. Gulf Coast toads can be identified by their parietal cranial crests, lines of warts on either side of the toads' bodies with dark coloration below the lines and a light stripe above them, as well as a light stripe down the spine. Males of the species are smaller than the females. They also have a yellow green throat and a nuptial pad on their front feet for amplexus (Porter 1970). Gulf coast toads are mostly nocturnal with both aquatic and terrestrial life stages. Like most anurans, *I. valliceps* relies on vocal calls to locate mates. They are also bioindicators due to the fact that they respire through skin. Gulf Coast toads are abundant in this area of central Texas. They are large enough to produce a sufficient amount of urine, which is required for hormone analysis. This species is also relatively easy to catch. Therefore, this species was selected for this study.

Hormones:

The second part of this study is a hormone analysis of Gulf Coast toad urine. This part of the study is currently being done. We are walking through Wild Basin looking for toads. Once we catch a toad, we use a pipette to extract urine from its cloaca. The cloaca or vent is the exterior hole used to release waste. We also weigh, measure snout to vent length, and sex the toads as well as take the location's coordinates and measure sound level in decibels. Additionally, we are recording the time and weather conditions.

Each urine sample collected is being stored at St. Edward's University in a -80 degrees Celsius freezer. When we have at least thirty, well distributed samples we will use a Corticosterone ELISA and a Urinary Creatinine kit to measure corticosterone concentrations in the urine. Once we get these measurements from the spectrophotometer, we will run a multiple regression test on the data. So far we have caught four toads.

Results:

The map we created in ArcGIS is shown in Figure 1. It shows the gradient of anthropogenic noise levels throughout the preserve. Noise was caused by mostly traffic but there was also eleven sound readings that included airplanes and three instances caused by visitors. We believe the map provides visual support of our first hypothesis which states that sound levels will decrease as distance from the highway increases.

We ran a regression analysis test on the sound data. The results showed that the average sound in decibels at each point is negatively correlated with its distance from TX-360 in meters (Figure 2). This correlation is statistically significant with a p-value that is

less than 0.001 and an r^2 of 0.661. This means that 66.1% of the variation in sound intensity within the preserve is explained by distance from the highway.

We currently do not have any results for the hormone testing portion of this study. We plan to perform the tests in 2016. By then we should have a sufficient amount of toad urine samples. We will then run a multiple regression test on the data including sound intensity, corticosterone levels, mass, snout to vent length, and sex.

Figure 1: This map shows the gradient of average sound intensity from the Capital of Texas highway throughout Wild Basin Wilderness Preserve. The shades of color show different ranges of average sound intensity in decibels. The lighter the color the lower the sound intensity levels.

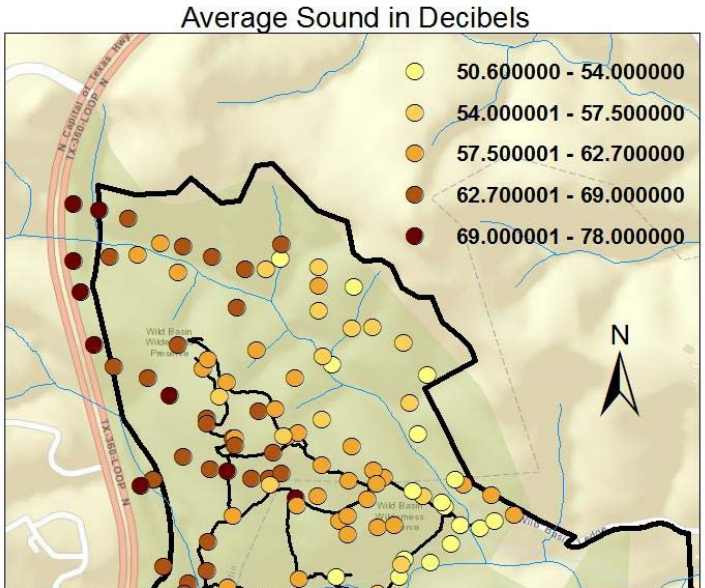
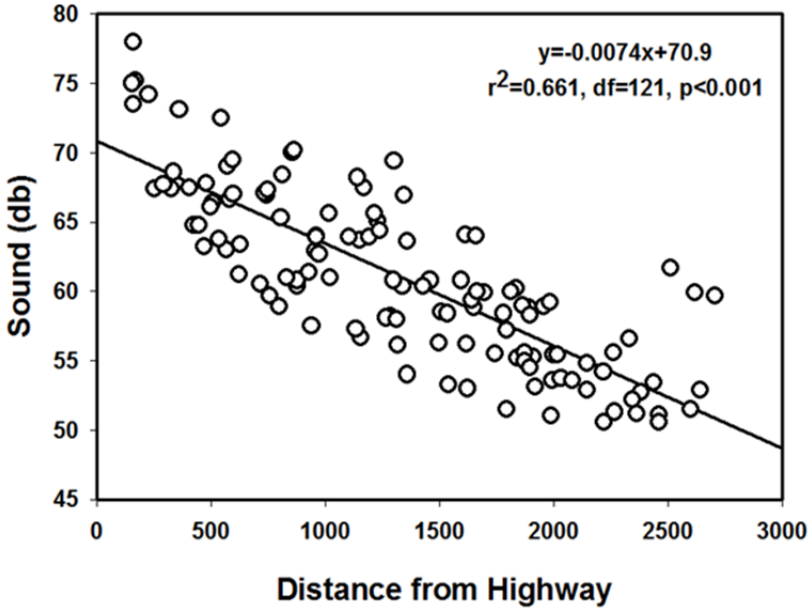


Figure 2: This graph shows the negative correlation between average sound intensity and distance from the Capital of Texas highway in meters. There are 123 points shown here. The equation for the linear trend line is f(x) =-0.0074x+70.9, the r^2 value is 0.661, and the p-value is less than 0.001 which can be seen the top right corner of the graph. The degrees of freedom is also shown.



Discussion:

The study of soundscapes is vital to understanding the acoustic ecology of an area and how noise pollution can affect it. Noise pollution is just one of the anthropogenic pollutants that can cause stress in most organisms. Consistent exposer to pollutants such as noise can cause chronic stress. Chronic stress can lead to multiple negative effects in these organisms. One of those effects is an increased susceptibility to infections (Goodenough et. al. 2010c). Therefore, by reducing the stressors of these organisms we can improve their ability to survive and reproduce.

This study has shown that anthropogenic noise can penetrate significantly into a wilderness preserve, showing that about two thirds of the variation of sound intensity within Wild Basin is caused by traffic noise. The effects of this "noise pollution" in a natural environment are not well known. However, lab experiments have shown that road noise can significantly increase stress hormone levels in frogs (Tennessen et. al. 2014). If noise pollution does have a significant impact on the health of organisms within a community, it may be possible to reduce the impact of human populations on natural communities. The effects of traffic noise on human populations from highways, railroads, or airports is a significantly more studied topic than that of the effects on anurans. Noise pollution has multiple social and economic consequences (Westman & Walters 1981; Harnapp & Noble 1987). Residential zones near high traffic areas commonly use sound barriers to refract noise away from homes. These sound barriers can be used in conjunction with native trees

for aesthetic purposes but also to increase the effectiveness of the sound barrier (Van Renterghem & Botledoorem 2002). Although the economic cost of a sound barrier is high, the potential to increase the economic value of a preserve, by preserving its ecosystem services, also has value.

Conclusion:

Distance from TX-360 was negatively correlated to sound intensity within Wild Basin Wilderness Preserve, with two thirds of sound variation throughout the preserve being explained by TX-360. The remainder of variation can be attributed to factors like tree density, differential elevation, or normal decay of sound intensity in a non-vacuum environment (Iglesias et. al. 2012).

With that, it is reasonable to ask how soundscape affects stress in organisms submitted to anthropogenic noise pollution. In anurans, lab studies have established that road traffic noise does elevate corticosterone levels (Tennessen et. al. 2014). Can similar results be found in nature? If we identify noise pollution as a source of stress in anurans, wildlife and preserve management methods can be altered to decrease anthropogenic effects on natural environments. By understanding yet another human variable that negatively impacts wildlife a more thorough effort can be made to slow the rate of global biodiversity decline, specifically in amphibian populations.

Ethics:

The methods for this study received IACUC approval from St. Edward's University. We also received a scientific research permit from Travis County, Texas allowing us to perform our study in Wild Basin Wilderness Preserve. The methods used for collecting and measuring hormones from urine are non-invasive for both captive and wild specimens and follow the methods by Narayan 2013 and Narayan et. al. 2010.

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